

CLAIMS

We claim:

1. A composition comprising a cleavage structure, said cleavage structure comprising:

- 5 a) a target nucleic acid, said target nucleic acid having a first region, a second region, a third region and a fourth region, wherein said first region is located adjacent to and downstream from said second region, said second region is located adjacent to and downstream from said third region and said third region is located adjacent to and downstream from said fourth region;
- 10 b) a first oligonucleotide complementary to said fourth region of said target nucleic acid;
- 15 c) a second oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said third region of said target nucleic acid; and
- 20 d) a third oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said second region of said target nucleic acid.

2. The cleavage structure of Claim 1, wherein said first region of said target nucleic acid has a length of eleven to fifty nucleotides.

3. The cleavage structure of Claim 1, wherein said second region of said target nucleic acid has a length of one to three nucleotides.

4. The cleavage structure of Claim 1, wherein said third region of said target nucleic acid has a length of six to nine nucleotides.

5. The cleavage structure of Claim 1, wherein said forth region of said target nucleic acid has a length of six to fifty nucleotides.

5 6. The cleavage structure of Claim 1, wherein one or more of said first, said second, said third and said fourth oligonucleotides contain a dideoxynucleotide on at the 3' terminus.

10 7. The cleavage structure of Claim 1, wherein said target nucleic acid is not completely complementary to at least one of said first, said second, said third and said fourth oligonucleotides.

8. The cleavage structure of Claim 7, wherein said target nucleic acid is not completely complementary to said second oligonucleotide.

9. A method of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

15 a) providing:

- i) a cleavage means,
- ii) a source of target nucleic acid, said target nucleic acid having a first region, a second region, a third region and a fourth region, wherein said first region is located adjacent to and downstream from said second region, said second region is located adjacent to and downstream from said third region and said third region is located adjacent to and downstream from said fourth region;
- 20 iii) a first oligonucleotide complementary to said fourth region of said target nucleic acid;

iv) a second oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said third region of said target nucleic acid;

iv) a third oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said second region of said target nucleic acid;

b) mixing said cleavage means, said target nucleic acid, said first oligonucleotide, said second oligonucleotide and said third oligonucleotide to create a reaction mixture under reaction conditions such that said first oligonucleotide is annealed to said fourth region of said target nucleic acid and wherein at least said 3' portion of said second oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' portion of said third oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products, each non-target cleavage product having a 3'-hydroxyl group; and

c) detecting said non-target cleavage products.

10. The method of Claim 9, wherein said cleavage means is a structure-specific nuclease.

11. The method of Claim 10, wherein said structure-specific nuclease is a thermostable structure-specific nuclease.